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The first record of the family Piophilidae (Insecta: Diptera) from the Caucasus

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Abstract

Piophilidae Macquart, 1835, is a small family of flies widely distributed in all parts of the world but more represented in the cooler temperate regions of the Northern Hemisphere. At present, there is a lack of information on the distribution of this family in the Caucasus region. In this study, the family Piophilidae, with a record of *Prochyliza nigrimana* (Meigen, 1826), is reported for the first time from the South Caucasus ecoregion.

Key words

CaBOL, DNA barcoding, Georgia, new record, *Prochyliza nigrimana*, South Caucasus

Introduction

Piophilidae (Insecta: Diptera) is a small family of small, rather stout, dark flies (Zuska 1984) belonging to the superfamily Tephritoidea (Bayless et al. 2021). The family includes two subfamilies, Neottiophilinae and Piophilinae (McAlpine 1977), the latter with three tribes: Piophilini, Mycetaulini, and Thyreophorini (Muller 2021). To date, there are more than 80 species of piophilids worldwide, of which about 40 occur in the Palearctic region (Pape et al. 2009). Although most Piophilinae adults are metallic blue or black in color and the body size typically ranges from 2.5 to 4.5 mm long, the members of the subtribe Mycetaulini are mostly brownish or orange, as are some Thyreophorini (McAlpine 1977; Barták 2009; Byrd and Castner 2010). The larvae feed on animal matter such as dried fish, carrion, and some types of cheese, the best-known example being the cosmopolitan cheese skipper *Piophila casei* (Linnaeus, 1758), which seriously harms the food industry, in particular cheese and meat (Byrd and Castner 2010). Although they are known to feed on dead or decaying matter (mushrooms, carcasses, etc.), there are also nest ectoparasites of birds such as *Neottiophilum praeustum* (Meigen, 1826), which lives in bird nests and feeds on the blood of nestlings (McAlpine 1977; Misiachna and Korneyev 2015). As Piophilidae are often found developing in carrion, including human corpses in various stages of decomposition (Velásquez et al. 2010), their importance in forensic science cannot be underestimated (Cockburn et al. 1975; Martín-Vega 2011).

As one of our planet's biodiversity hotspots, there is a significant knowledge gap in the Caucasus biodiversity (Wetzel et al. 2018; Mumladze et al. 2020), and the dipterans of this region are poorly studied compared to other regions (Oboňa et al. 2019). Nevertheless, taxonomic research in recent years on the dipteran fauna of the Caucasus has proved fruitful, resulting in several new records and species descriptions from the region and especially from Georgia (Nartshuk 2004; Pont 2012; Oboňa et al. 2019; Mengual et al. 2020; Negrobov et al. 2020; Evstigneev and Glukhova 2022; Whittington and Beuk 2022). Part of these efforts are framed by the Caucasus Barcode of Life (CaBOL) project (https://ggbc.eu/). The CaBOL project aims to gather new data for the Caucasus region and provide an accurate DNA-based library for all

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living organisms in the region (Thormann et al. 2019). The project was launched in 2018, and since then we have gathered data for 19,241 specimens of 3,396 species (https://ggbc.eu/data/sammeln/factsheet).

To our knowledge, the Piophilidae have not been recorded in the Caucasus so far (Zuska 1984). Misiachna and Korneyev (2015) listed *Mycetaulus bipunctatus* (Fallén, 1823) and *Stearibia nigriceps* (Meigen, 1826) from the Caucasus, referring to Ozerov (1999) and Duda (1924), respectively. The original text of Ozerov (1999: page 535) refers to the European parts of Russia and does not explicitly mention the Caucasus, while Duda (1924) lists material of *S. nigriceps* in the Naturhistorisches Museum Wien (Vienna, Austria) from the "Kaukasus" without further details. Thus, the individual reported here is the first reliable finding of this family in the Caucasus.

Materials and methods

Sample collection and processing

The material examined was collected using a sweepnet in a meadow next to Sakisto Lake (Tbatana Lake, Georgia) (Fig. 1). The collected specimen was then preserved in 96% ethanol and deposited in the collection of the Ilia State University, Institute of Ecology.

Morphological determination was carried out using the identification key of Martin-Vega (2014). In addition, the species identification was corroborated by Martin-Vega himself.

Digital photographs of the collected specimen CaBOL-ID 1028210 (Fig. 2) were taken using a Canon® EOS 60D camera with a Canon® MP-E 65mm f/2.8 1-5x Macro Photo lens. The final composed digital image was prepared using the Zerene® Stacker image stacking software and Adobe® Photoshop CS6.

Figure 1 was generated with QGIS desktop 3.16.3 software.

DNA processing

DNA was extracted from the whole specimen using the Quick-DNA Magbead Plus Kit (Zymo Research). Partial sequences of cytochrome oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pairs LCOI490-JJ and HCO2198-JJ (Astrin and Stüben 2008). Thermal conditions included denaturation at 95°C for 1 min, followed by the first cycle set (15 cycles): 94°C for 30 sec., annealing at 55°C for 1 min (–1°C per cycle), and extension at 72°C for 1:30 min. Second cycles set (25 cycles): 94°C for 35 sec., 45°C for 1 min, 72°C for 1:30 min, followed by 1 cycle at 72°C for 3 min and a final extension step at 72°C for 5 min. PCR amplicons were visualized on 1% agarose gels using 1.7 µl of PCR product. Sequencing of the unpurified PCR products in both directions was conducted at the Beijing Genomics Institute (Hong Kong, CN) by using the amplification primers. Sequence analysis was performed using Geneious Prime 2022.1.1 (http://www.geneious.com). The DNA was deposited in the scientific collections of Ilia State University, Tbilisi, Georgia, while the sequences were submitted to Barcode of Life Data System (BOLD) databases. The newly obtained DNA barcodes of COI sequences were checked against the BOLD Systems database (http://www.boldsystems.org/index.php). The Barcode Index Number (BIN) (Ratnasingham and Hebert 2013) for the sequenced taxa and for their nearest neighbor in BOLD systems (if they had a BIN) are also given. For the calculation of sequence differentiation, we used uncorrected *p*-distance as performed in BOLD.

Results

Family Piophilidae Macquart, 1835

Prochyliza nigrimana (Meigen, 1826)

Material examined. Georgia • 1 ♂; Tbatana; N42.287017°, E45.242762°; 2059 m a.s.l.; 29 May 2022; leg. CaBOL members (IZ ISU); meadow next to the lake; CaBOL-ID 1028210 (Fig. 2).

Genetics. The newly obtained COI sequence for the specimen CaBOL-ID 1028210 (BOLD:ADL1379) is 658 bp long and is nearly identical to the 5'-end COI sequence of *P. nigrimana* from Spain in GenBank (Accession: OM791734, *p*-distance = 0.5%). There are 7 sequences available for this species in the BOLD Systems database - all 483 bp long except for one that has a length of 349 bp. A comparison of the obtained sequence with those in BOLD systems failed to determine the species, probably due to the significant length differences.

Discussion

In this study, the first record of the family Piophilidae and *P. nigrimana* from Georgia (and the South Caucasus ecoregion) is provided.

According to McAlpine (1977) and Martin-Vega (2014), the genus *Prochyliza* Walker, 1849 (Diptera: Piophilidae) comprises the following species: *P. azteca* McAlpine, 1977, *P. brevicornis* Melander, 1924, *P. georgekaplani* Martín-Vega, 2014, *P. inca* McAlpine, 1977, *P. lundbecki* (Duda, 1924), *P. nigricoxa* (Melander and Spuler, 1917), *P. nigrimana* (Meigen, 1826), and *P. xanthostoma* Walker, 1849.

Described from Germany (Meigen 1826), *Prochyliza nigrimana* occurs throughout the Holarctic and Neotropical Regions and is the most geographically widespread species among the congeners: it has been commonly cited throughout Europe, including the Azores and the Canary Islands, the Middle East, and North America (Duda 1924; Hennig 1943; Zuska and Laštovka 1965; McAlpine 1977; Ozerov 1999; Martin-Vega 2014), as well as South America, where it appears to have been introduced by humans (McAlpine 1977; Ozerov and Norrbom 2010). The species displays seasonal polymorphism (Martin-Vega and Baz 2011; Martin-Vega et al. 2012). Both morphological and molecular characters agree on the identification of the male specimen collected at Sakisto Lake.

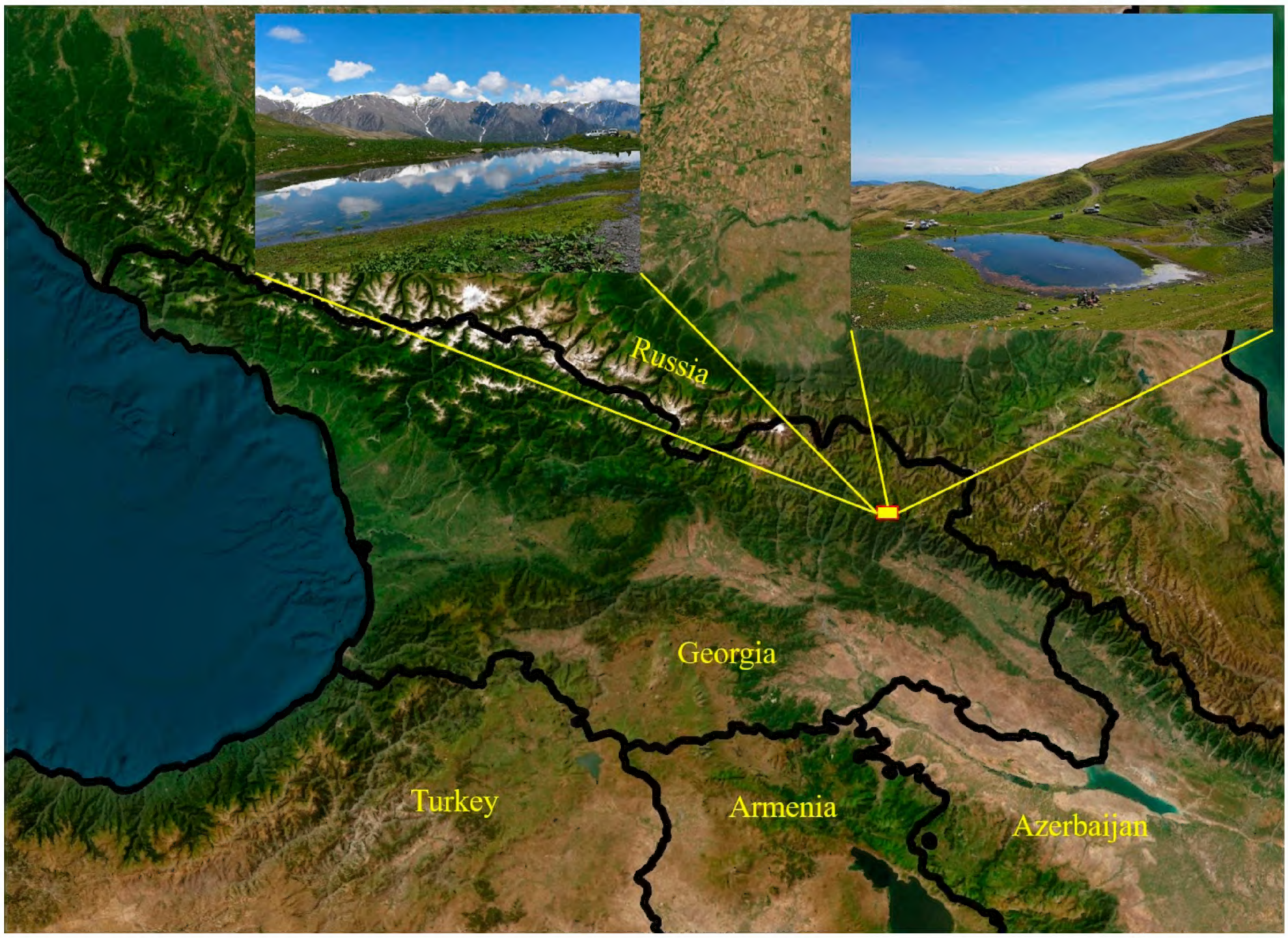

Figure 1. Sakisto Lake (Tbatana Lake), Georgia, showing collecting site of *Prochyliza nigrimana*, presented as the yellow dot square (ESRI Satellite, ArcGIS/World_Imagery).

Figure 2. *Prochyliza nigrimana*, Georgia, Sakisto Lake (Tbatana), CaBOL-ID 1028210 (Photo credit: Armen Seropian).

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